PATENT COOPERATION TREATY

From the:
INTERNATIONAL SEARCHING AUTHORITY

To:

Griffith Hack
GPO Box 1285K
MELBOURNE VIC 3001

GRIFFITH HACK
- 1 MAR 2005
1. Lee
2.
3.

PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)*	28 FEB 2005
Applicant's or agent's file reference JSB:SP:FP20705	FOR FURTHER ACTION See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/AU2004/001577	15 November 2004	14 November 2003

International Patent Classification (IPC) or both national classification and IPC
Int. Cl. [7] C12N 1/00; 1/20, 1/26, 1/38.

Applicant
COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANISATION et al

1. This opinion contains indications relating to the following items:

- [X] Box No. I — Basis of the opinion
- [ ] Box No. II — Priority
- [ ] Box No. III — Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- [ ] Box No. IV — Lack of unity of invention
- [X] Box No. V — Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- [ ] Box No. VI — Certain documents cited
- [ ] Box No. VII — Certain defects in the international application
- [X] Box No. VIII — Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the IPEA/AU	Authorized Officer
AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	**GILLIAN ALLEN** Telephone No. (02) 6283 2266

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Box No. I Basis of the opinion

1. With regard to the **language,** this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This opinion has been established on the basis of a translation from the original language into the following language , which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material

      ☐ a sequence listing

      ☐ table(s) related to the sequence listing

   b. format of material

      ☐ in written format

      ☐ in computer readable form

   c. time of filing/furnishing

      ☐ contained in the international application as filed.

      ☐ filed together with the international application in computer readable form.

      ☐ furnished subsequently to this Authority for the purposes of search.

3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

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Box No. V **Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims	**3-5, 7-9, 15-18, 21, 24-25**	**YES**
	Claims	**1-2, 6, 10-14, 19-20, 22-23**	**NO**
Inventive step (IS)	Claims		**YES**
	Claims	**1-25**	**NO**
Industrial applicability (IA)	Claims	**1-25**	**YES**
	Claims		**NO**

2. Citations and explanations:

Citations

D1 Acha, V.; Meurens, M.; Naveau, H.; Agathos, S. N. Detoxification of a mixture of aliphatic chlorinated hydrocarbons in a fixed-bed bioreactor : continuous on-line monitoring via an attenuated total reflection-Fourier transform infrared sensor. Water Science and Technology (1999), 40(8), 41-47.

D2 Stuart S L; Woods S L. Kinetic evidence for pentachlorophenol-dependent growth of a dehalogenating population in a pentachlorophenol- and acetate-fed methanogenic culture. Biotech and Bioeng, 1998. 57(4): 420-429.

D3 Bellco CellTrol II Control Modules
http://www.bellcoglass.com/us/7803-81102.shtml. 31 August 2003. http://www.archive.org/ used to establish the publication date of the document.

D4 BioNet Utility Tower (Single, Dual, or Quad)
http://www.broadleyjames.com/bionet-tower.html. 2 October 2003. http://www.archive.org/ used to establish the publication date of the document.

Novelty

The invention is directed to methods of enriching chemostat/bioreactor cultures for microbes exhibiting a desired biochemical activity, where there is continuous on-line monitoring of a parameter relating to success of the enrichment proceedings. The signal output from the monitor can be used to regulate culture conditions, specifically nutrient and substrate flow rate.

D1, Acha et al, discloses adaptation (ie enrichment) of a biological consortium to a mixture of TCE, PCE, CT and HCB in a bioreactor. The reaction is monitored on-line via an attenuated total reflection-fourier transform infrared sensor to track concentrations of the chlorinated substrates. Monitoring is continuous. The citation discloses flow of nutrients and chlorinated substrates through the bioreactor. Since the chlorinated substrates are being metabolised, clearly measurement of substrate level and its rate of disappearance must be considered measurement of metabolic indicators.

Claim 1-2, 6, 10-14, 19-20, 22-23 are not novel or inventive over D1.

Continued in Supplemental Box V(i)

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Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims; description, and drawings or on the question whether the claims are fully supported by the description, are made:

Claim 19 is not clear.

I cannot determine what range of substrates the term "not a commonly metabolised substrate" might encompass. Nor can I determine at what point a substrate can be considered as "not commonly metabolised" in terms of any measurable quantity, such as the percentage of known microorganisms that metabolise the substrate.

Supplemental Box V(i)

In case the space in any of the preceding boxes is not sufficient.

Novelty (cont'd)

D2, Stuart et al, discloses enrichment of a dechlorinating bacterial population metabolising PCP in a computer-monitored/feedback-controlled continuous flow bioreactor. The reactor featured continuous monitoring of pH, sulphide, redox potential, and feedback controlled maintenance of pH coupled to acetate maintenance.. It is disclosed that acetate consumption is directly related to increased biomass. Acetate uptake is a metabolism indicator monitored on-line by pH.

Claims 1-2, 10, 14, 19, 20, 22, 23 are not novel or inventive over D2.

Inventive Step

The problem addressed by the invention is to provide improved methods for obtaining enriched microorganism cultures that have desirable metabolic properties, either from mixed microbial populations or from populations of a single type of microbe.

The solution provided by the applicants, as defined by the claims, is to provide microbial cultures within an environment having on-line monitoring systems that enable monitoring of the enrichment process via monitoring of a metabolic indicator, and controlled nutrient/substrate flow capacity, wherein the culture medium comprises the substrate, ie the substance to be metabolised.

I cannot determine wherein lies any inventive step in the invention as defined by the present claims.

It is standard methodology to select for biological organisms that have desirable metabolic traits by exposing them to selection pressure by including the desired metabolite/substrate in the biological environment, often at gradually increasing concentrations. In mixed microbial cultures it is well-known to enrich the culture for microbes having the desired metabolic trait by including the metabolite/substrate in the culture. If the metabolite has a suitable chemical structure, it may be provided as the sole carbon or nitrogen source. It is, moreover, standard methodology to monitor such cultures by any of a variety of methods. It is noted that any biochemical activity of the microbe would be regarded as metabolic. It is thus difficult to consider any signal from the bioreactor, able to monitor changing conditions within the bioreactor, that would not be regarded as a metabolism indicator. On-line monitoring has obvious convenience, and commercially available on-line systems for analysis of a variety of chemical compounds, or determination of a variety of optical parameters (eg spectrophotometry, fluorimetry) exist. Such on-line analysis systems could be provided to analyse chemostat outputs without requiring inventive skill.

It is also considered that one skilled in the art would expect to, and have the skills to, adjust nutrient and substrate flows etc to optimise culture conditions within the chemostat.

Claims 3-5, 7-9, 15-18, 21, 24-25 are not inventive over D1 or D2 as they are merely directed to methods of optimising culture conditions, or monitoring parameters, other than those disclosed in the citations. Neither of these is considered to provide invention, as there is no disclosure of specific technical problems that had to be overcome, or any surprising or unexpectedly advantageous result.

Continued in Supplemental Box V(ii)

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Supplemental Box V(ii)

In case the space in any of the preceding boxes is not sufficient.

Continuation of: **Inventive Step**

D3 and D4 disclose commercially available bioreactor systems with computer control modules.

D3 discloses on-line monitoring of bioreactor signals, including dissolved oxygen, temperature, weight and tachometer readings. The system provides different selectable pump speeds and gas flow. The system can be configured with a variety of other analog probes as well as oxygen probes.

D4 discloses online monitoring of pH, dissolved oxygen and temperature measurements, and variable speed pumps, and discloses that the bioreactor conditions may be preset. D3 and D4 enable all the reaction conditions and monitoring systems of the claims, and would have obvious use in any known procedures for rapidly providing and assessing microbial enrichment cultures.

Because methods of enriching microbial cultures for desired metabolic traits are so well known in the art, they cannot provide invention in the absence of unexpected results or technical problems overcome by inventive means. Therefore, all claims are considered to lack inventive step over the prior art of D3 and D4.

NOTE: It is noted that the applicants have stated , p3 of the description, that the "present inventors have found that the above method (as defined by claim 1) for on-line determination of a change in the metabolism indicator, such as O^2, as an indicator of cellular activity enables indirect measurement of biomass or substrate utilisation and have identified that this can be used to assess the status of a population of microorganisms in real time. If the applicants' invention is related to this "discovery", it is noted that the claims are not limited to methods based on this discovery. Therefore this aspect of the invention has not been searched by the ISA, and no opinion can be offered as to its inventiveness.